مجلة العلوم البحتة والتطبيقية
Journal of Pure & Applied Sciences
www.Suj.sebhau.edu.ly ISSN 2521-9200
Received 04/05/2019 Revised 10/09/2019 Published online 17/11/2019

Risk factors of toxoplasmosis in Libya: a brief review

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Abstract Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii* which infects both humans and animals as a zoonotic pathogen wide spread in nature. Infection in humans occurs worldwide, but prevalence varies significantly between populations. In Libya, *T. gondii* is prevalent among humans as well as animals, however, the main reason yet unclear as no adequate studies regarding transmission modes were available. Thus, the present review aimed to collect and summarize data on the risk factors associated with of *T. gondii* infection in Libya. Published data from national and international databases were reviewed. Prevalence of *T. gondii* infection among humans and animals were also discussed. This review would be a useful tool for proposing appropriate national toxoplasmosis control programs.
Keywords: toxoplasmosis, *Toxoplasma gondii*, risk factors, Libya.

عوامل الخطورة المرتبطة بالإصابة بداء المقوسات في ليبيا

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الملخص داء المقوسات هو مرض يسببه طفيل أولي يعرف بالمقوسة القوندية *Toxoplasma gondii* الطفيل يصيب كل من الإنسان و الحيوانات كطفيل حيواني المنشأ واسع الانتشار حول العالم. الإصابة شائعة في جميع أنحاء العالم، و لكن معدل الانتشار يتباين بين المجتمعات المختلفة. المقوسة القوندية واسعة الانتشار في ليبيا و تصيب كل من البشر و الحيوانات و لكن الأسباب الرئيسة لانتشار المرض غير واضحة لعدم توفر دراسات وافية حول طرق انتقال الإصابة. لذلك تهدف هذه الدراسة إلى جمع و تلخيص المعلومات المتوفرة حول عوامل الخطورة المرتبطة بالإصابة بداء المقوسات في ليبيا. تم استعمال المعلومات المنشورة بقاعدات البيانات الوطنية و العالمية، كما تمت مناقشة انتشار داء المقوسات بين الإنسان والحيوانات. وبذلك فإن هذه الدراسة هي أداة مفيدة يمكن استعمالها لاقتراح برامج وطنية مناسبة لمكافحة داء المقوسات Toxoplasmosis.

الكلمات المفتاحية: المقوسة القوندية، داء المقوسات، عوامل الخطر، ليبيا.

Introduction

Toxoplasmosis is an important zoonotic disease caused by an obligate intracellular protozoan parasite called Toxoplasma gondii [1,2]. The parasite is able to infect humans as well as other warm blooded domestic and wild animals [3,4]. All mammals, including humans, and birds are intermediate hosts, whereas Felidae (cats) are definitive as well as intermediate hosts [1]. *T. gondii* was first described in Tunisia by Nicolle and Manceaux at the Pasteur Institute in 1908. Whilst conducting leishmaniasis research on the North African rodent, Ctenodactylus gondi, the investigators isolated *T. gondii* merozoites from its blood, liver and spleen. In 1923, Janku described the parasitic cysts in the retina of a child with congenital hydrocephalus which considers first infection by toxoplasmosis in human [2]. In 1939, Wolf and colleagues successfully isolated the parasite from tissue from a neonate with encephalitis [5,6]. Toxoplasma has a number of genetic types but about 95% of them are categorized into three classes of strains simply named as Type I, II and III. These genotypes are considered to be clonal in their structural features [7]. Type I causes infection in rodents. Type II has been established as the infectious agent of toxoplasmosis in small ruminants while Type III has yet not been proved as infectious strain. All the three genetic types I, II and III can cause toxoplasmosis in human. Recently, a newly discovered genotype (Type IV) has also been reported mostly in wildlife [8]. Human infections are acquired through direct or indirect contact with cat feces. Transmission of the parasite can occur in several ways, by eating raw or undercooked meat containing *T. gondii* tissue cysts, ingesting oocysts via contact with infected cat feces, or by touching contaminated soil or consuming food or water contaminated with oocysts [9], or by acquiring congenital infection through the placenta [10]. In addition, *T. gondii* infection can also be acquired by blood transfusion and organ transplantation [4,11]. The overall life cycle of Toxoplasma contains two distinct cycles: a sexual that occurs in the small intestine of the feline family (definitive hosts), and an asexual phase in infected animals, including humans (intermediate hosts). [6,12]. The parasite has three infective forms during its life cycle: tachyzoite, bradyzoite, and oocysts . However, all hosts, including humans, can be infected by any one of the three forms of the parasite. The rapidly dividing Tachyzoite forms

found during the acute stage of infection which are capable to invade and replicate within cells and are responsible for congenital infection. The slowly dividing bradyzoite found during latent infections; this form of the parasite is present in tissue cysts. Oocysts are the zygotic stage of the life cycle, and are excreted unsporulated in cat faeces. Sporulation occurs outside of the body to form sporulated oocyst containing four sporozoites. The oocysts are environmentally robust, and can retain infectivity in a cool damp environment for months [3,13]. Primary infection of toxoplasmosis in healthy individuals is normally asymptomatic or associated with influenza-like illness, which including limited symptoms such as fever , myalgia, malaise, and headache [14,15,16]. However, toxoplasmosis can cause serious implications in immuno-suppressed individuals including HIV patient, pregnant women as well as its severe consequences on fetuses in congenital transmission [17]. The disease in pregnancy has been associated with spontaneous abortion, miscarriage, stillbirth, hydrocephalus, intrauterine malformations in the fetus, cerebral calcification and chorioretinitis in the newborn [17,18].

Diagnosis of toxoplasmosis in humans is performed using different techniques including serological testing, histological identification, isolation in tissue culture as well as, molecular methods using the Polymerase Chain Reaction (PCR) or by a combination of these techniques. Acute and latent *T. gondii* infections are mostly diagnosed by serological tests including increased antibody levels such as IgG, IgM, IgA and IgE. In a primary *T. gondii* infection, IgM appears a few weeks after infection, followed by IgA and IgE. These acute phase immunoglobulins peak after about two months and are usually undetectable by serological tests by six to nine months but can persist for longer periods of time. IgG appears after IgM peaks after four months and persists at low levels throughout the duration of the host's life [6,16]. the most commonly used serological tests include the indirect haemagglutination assay, indirect fluorescent antibody assay (IFA), direct agglutination test (DAT), Latex agglutination test (LAT), and Enzyme-Linked Immunosorbent assay (ELISA) [19].

Risk factors associated with transmission of *Toxoplasma gondii*

T. gondii is prevalent in Libya in both humans and animals [20,21]. Several studies have been conducted on women in childbearing age (pregnant women, non-pregnant), psychosocial patients ,schoolchildren and blood donors to determine the prevalence of infection. Most of the studies were carried out in the east, west and north of Libya, whereas few studies conducted in the south. The results showed significant differences from region to region (Table 1). The main source of infection to herbivores is ingestion of oocysts contaminated in grasses, feed and water. Therefore, toxoplasmosis in animals is significantly associated with presence of cats in the farms [7]. Most of the acquired infections of *T. gondii* in herbivores (Sheep, goat, cattle, camle and equine) are subclinical. However, fever, ataxia and retinal degeneration and encephalomyelitis may develop. Severity of toxoplasmosis in ovine is associated with the stage of pregnancy[22]. The most devising outcome of toxoplasmosis is miscarriage or abortion which is particularly important in humans and domestic livestock. Additionally, it can cause a wide variety of neurological disease especially when transmitted congenitally. In Libya, limited studies are made on the prevalence of toxoplasmosis among animals. El-Gomati et al (2008 and 2010) [24.25] found 40.7% and 35% *T. gondii* infection among sheep and mice in Tripoli respectively.

(Table 1) Prevalence of T. gondii in Libya

Region/ Year	Sample size	Population	Assay	Seroprevalence (%)	Reference
Tripoli 1987	2000 300 1980	adult males adult females schoolchildren	Latex Agglutination (LA)	51·6 43·4 43·7	[44]
Benghazi 1991	369	pregnant women	indirect haemagglutination test (IHA)	47.4	[33]
Tripoli 2008	692	women who suffered spontaneous abortion	ELISA	IgM 17.6, IgG 45	[45]
Tripoli 2008	474	non-pregnant	ELISA	IgG 18.14	[46]
Benghazi 2011	143	pregnant women with previos adverse pregnancy outcome.	ELISA	IgM 8.4, IgG 44.8	[39]
Tripoli 2014	300 300	psychiatric patients control volunteers	Latex ELISA Latex ELISA	61.7 IgG 50.3 46.7 IgG 33	[31]
Benjawad 2015	280 250	pregnant non-pregnant	ELISA	IgM 3.57, IgG 37.14 IgM 3.60, IgG 37.20	[32]
Alkhoms 2015	361	pregnant women	ELISA	IgG 39.3	[36]
Misurata 2015	300	pregnant women	Latex Elecsys-Cobas e analyzer	26.7 IgM 1.00, IgG 26.7	[47]
Sebha 2016	267	pregnant women	ELISA	IgM15.37,IgG 25.89	[49]

Sebha 2016	190	pregnant women	ELFA	IgM 0.00, IgG 36.84	[30]
Tripoli 2016	140 26 9 2	abortions women. HIV-positive patients. patients with leukemia and lymphoma. infants with ocular infection	ELISA	38.5 88 66.6 50	[21]
Tripoli 2017	289 92 56 150	children suspected cryptogenic epilepsy. symptomatic epilepsy non-epilept.i	ELISA	IgG 35.2 IgG 29.4 IgG 29	[37]
Msallata 2017	170	abortions women.	latex agglutination test ELISA	41 IgM 35, IgG 26	[50]
Benghazi 2018	43	patients clinically suspected of ocular toxoplasmosis.	ELISA	IgM 0.00, IgG 55.8	[48]
Benghazi 2018	200 50	Type-2 Diabetic Patients. non-diabetics individual.	ELIS	IgM 10.5 , IgG 41.5 , IgA 3.5 IgM 4.00 , IgG 24.00 , IgA 0.00	[22]

Recently, Al-Mabruk *et al* (2013) [26],found significantly higher seroprevalence (71%) of antibody of *Toxoplasma* in sheep in Tripoli. They consider sheep are suitable host for *T. gondii* in libya. Worldwide, prevalence of the toxoplasmosis is measured by detection of specific anti-*Toxoplasma* IgG antibodies varies between 1% and 100 depending on different factors such as geographical location, age, habit of eating raw meat or unwashed fruit and vegetables, sociocultural and nutritional habits ,general level of hygiene and contact with domestic cats. [2,27, 28].

Geographical location: Prevalence of infection with *T. gondii* varies widely between countries and often from one country to another or even within the same country or between different communities in the same region. This wide variability may be attributed to differences in climatic conditions, cultural differences regarding hygienic and feeding habits. A higher sero-prevalence is associated with humid areas which are favourable for sporulation of oocysts that voided in cat feces compared to arid areas. Moreover, oocyst survival increases in humid conditions where it can remain viable in a moist environment for more than a year [29,30,31]. In Libya, results of several studies showed high prevalence of parasite in the eastern and western regions compared to the southern region of the country. This can be attributed to the difference in environmental conditions, such as the high rainfall, and high humidity, making it an environment suitable for the sporulation and surviving of oocysts. However, hot and dry environment as such in the southern regions of the country could be unfavoring the development and surviving of the parasite which may explain the moderate to low incidence of *T. gondii* in the southern regions [32].

Age: Previous studies conducted in Libya reported high rate of positivity of *T.godii* among the older age group [20,30,31,32,33,34,35, 36,37]. This association does not mean that older age is a risk factor predisposing to infection but might be explained by the older the person the longer time being exposed to the causing agent and may retain a steady level of anti-toxoplasma IgG in serum for years [36]. On contrary, results of studies done Benghazi and Sebha Cites showed a clear declination of seropositivity with age [22,38, 49]. This difference may be due to wide variations in age groups used in these studies. This could be explained by that older women are more likely to have been exposed to any one of the risk factors than younger women as a result of longer exposure time. In addition, decrease of immunity in old ages; increase the chance for more exposure to infection [30,32].

Consumption of undercooked meat: Meat of warm-blooded animals and birds has been considered a major source of *Toxoplasma* infection especially in countries that consumed raw or undercooked meat. Besides the consumption of *T. gondii* tissue cysts contained in meat, meat-derived products, or offal can be an important source of infection in humans [29,30]. Virtually, all edible portions of an animal can harbor viable *T gondii* organisms. It can also be transmitted by containers, knives or other utensils, cutting boards or other preparation surfaces contaminated with raw meat. Touching unwashed hands to the face after meat preparation is another source of infection. Type of meat consumed should also be considered since pork, mutton and farm chickens are commonly infected, while beef meat are rarely infected [30]. In their study, Gamal & Jaroud (2015) [36], reported significantly higher seropositvity rate (70.0%) among women consuming sheep meat compared to those (54.0%) consuming cow meat. The method and degree of meat cooking have an effect on seropositivity to *T.gondii*. Previous studies have shown that people who consuming undercooked meat have higher risk of infection than those eating well cooked meat [36].In a study conducted by El-sayed *et al.* (2016), the sero-positivity of *T.gondii* was high among pregnant women eating roasted mutton or processed meat as hamburger, minced meat and Sharrma which may be insufficiently cooked [30,36]. Raw or undercooking meat consumption was positively associated with *T.gondii* infection. Several studies identified an association between eating raw meat and *T.gondii* seropositivity [37,39].

On the other hand, transmission of *T.gondii* infection through uncooked meat in Libya is uncommon because most people preferred to eat well cooked meat [30,34,40].Generally, thorough cooking is always preferred in Libya, and therefore the most possible way of transmission is probably through handling of raw contaminated meat, during food preparation. Local meat, sheep, goat or camel might become contaminated with oocysts due to poor hygiene during handling of meat from slaughter house to kitchen. In addition, in Libya, consumption of lamb is greater than that of beef. These trends may have increased exposure to toxoplasma because lamb has a higher risk of infection than beef or poultry [39].

Eating raw or unwashed fruit and vegetables: Contaminated fruit and vegetables by cat faeces have been considered source of *T. gondii* infection, especially in developing countries. This may attributed to their contamination with oocysts that carried by soil and water, in addition to, poor hygienic measures in these places suh as consmption of unwashed vegetables. Several previous studies [31,37,49] and have found an association between the seropositivity among personals and intake of raw vegetables if proper hygiene is lacking and ingestion of oocysts occurs. On contrary, results of other studies found no such association [30].

Drinking water: Drinking of contaminated water is another source of *T. gondii* infection as oocysts of *T. gondii* can remain infective in water for a long time (i.e., under optimal conditions several months or even years) [41]. They are not killed by chemical and physical treatments currently applied in water treatment plants, including chlorination and ozone treatment [42]. So, high prevalence was observed among people who drunk untreated water that may have high risk of contamination by oocysts [30]. In a study conducted by Gamal and Jaroud (2015), the higher prevalence rates of *T. gondii* were reported in women using rain water (68.0%) and well water (60.0%). Meanwhile lower prevalence was in women using general network water (33.0). Corresponding finding was reported by Elsaid *et al.* (2014) in psychiatric patients in Tripoli.

Consumption of unpasteurized milk: The infection via milk is not an important risk factor because tachyzoites which may pass in milk is very sensitive to environmental conditions and is usually killed rapidly outside the host. Also, tachyzoites were suggested to be rare cause of acquired toxoplasmosis in humans after the consumption of unpasteurized goat's milk. However, a significant association between the consumption of raw milk and infection was recorded [30]. Generally, most of Libyan population consumes pasteurized milk. However, the consumption of raw milk in rural and some suburban areas is common [37]thus, consumption of non-processed milk from several animals is a potential source of *T. gondii* transmission [43].

Contact with domestic cats: Contact with cats and cats excrement have been considered as major risk factors for acquiring infection as oocysts that voided in feces are main source of infection for human and animals [30]. The association between the infection and contact with cats was observed in several studies conducted in Libya [22,32,33,36.49]. On the contrary, other studies found no association between seroprevalence of toxoplasmosis and contact with cats. [20, 30,31.35,39, 37]. In addition, contact with dogs showed significant associated with infection; therefor dogs might be an important route of *T. gondii* transmission [31]. However, the acquisition of cats as pets is not common practice in Libya and most of cats are straying. Stray cats were found in farms, gardens or may enter houses from time to another to obtain their food from remains of raw meat thrown in the garbage. This could increase the chance of infection especially for children living in houses with soil floor or playing in farms during picnics [30,39]. Hence, the cats play a central role in the epidemiology of *T. gondii* and constituting the only known source of environmental contamination with the infective oocyst stage. A high risk is thus imposed on human communities that come into contact with cats [15]. Cats may also play indirect role in transmission of toxoplasma through contaminated meat, vegetables and fruits.

Contact with contaminated soil: The soil consider as risk factor for transmitting *T.gondii* due to their contamination by oocysts dispersed in the excrement of stray cats. Soil contaminated with cat's faeces may play role in transmitting infection through contamination of raw vegetables or water [30]. Therefore, contaminated fruit and vegetables by cat faeces and poor hand hygiene are important in parasite transmission [37].

Blood transfusion: Transmission of *T.gondii* through blood transfusion though uncommon, it is theoretically possible if the donor has recently acquired a *Toxoplasma* infection. However, several studies in libya showed no significant association between *T. gondii* seropositive and blood transfusion [30,37].

Conclusion:

Toxoplasma gondii is a widely prevalent parasite among both humans and animals that is potentially responsible for significant morbidity and mortality. While occurrence of the infection is well documented In Libya, the main sources of infection remain unclear. Based on available data, several risk factors of toxoplamosis in Libya were identified including contact with cat and, consumption of raw unwashed fruit and vegetables, consumption of undercooked or unhygienic prepared meat, drinking untreated water, older age, and geographic location. Therefore, knowledge of these risk factors could help to further reduce the burden of toxoplasmosis in Libya, raising health awareness, and designating of control strategies. Besides, attention should also be given to environmental sampling in order to develop adequate transmission models between animals, the environment and people. Furthermore, more comprehensive epidemiological studies are needed to guide decision makers to adopt and implement control programs involving both the medical and veterinary sectors.

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